

DENNY et al  
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 March 6, 2009

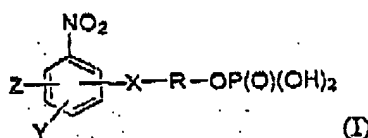
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# AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

1 (currently amended). A phosphate compound of Formula (I)



wherein:

X represents at any available ring position -CONH-, -SO<sub>2</sub>NH-, -O-, -CH<sub>2</sub>, -NHCO- or -NHSO<sub>2</sub>-;

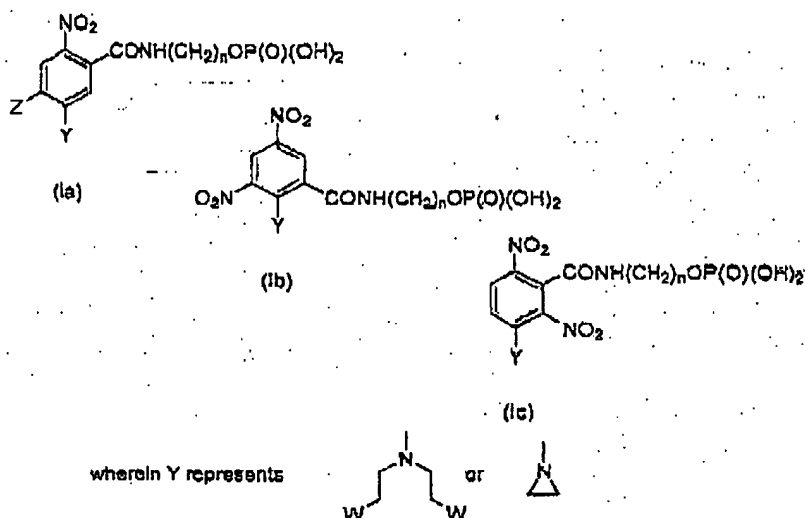
R represents a lower C<sub>1-8</sub> alkyl optionally substituted with one or more groups including selected from hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom;

Y represents at any available ring position -N-aziridinyl, -N(CH<sub>2</sub>CH<sub>2</sub>W)<sub>2</sub> or -N(CH<sub>2</sub>CHMeW)<sub>2</sub>, where each W is independently selected from halogen or -OSO<sub>2</sub>Me.

Z represents at any available ring position -NO<sub>2</sub>, -halogen, -CN, -CF<sub>3</sub> or -SO<sub>2</sub>Me; and pharmaceutically acceptable salts and derivatives thereof.

2 (original). A phosphate compound of Formula (I) as claimed in claim 1 which is selected from a compound represented by formulae (Ia), (Ib) or (Ic)

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and wherein

n represents 1 to 6

Z represents  $-\text{NO}_2$ , -halogen,  $-\text{CN}$ ,  $-\text{CF}_3$  or  $-\text{SO}_2\text{Me}$ ; and

where each W is independently selected from halogen or  $-\text{OSO}_2\text{Me}$

and pharmaceutically acceptable salts and derivatives thereof.

3 (previously presented). The phosphate compound of Formula (I) as claimed in claim 1 which is selected from:

2-[[2-[Bis(2-bromoethyl)amino]-3,5-dinitrobenzoyl]amino] ethyl dihydrogen phosphate;

3-[[5-[Bis(2-chloroethyl)amino]-2,4-dinitrobenzoyl]amino]propyl dihydrogen phosphate;

3-[[5-[Bis(2-bromoethyl)amino]-2,4-dinitrobenzoyl]amino]propyl dihydrogen

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phosphate;

2-[[2-[Bis(2-chloroethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl dihydrogen

phosphate;

2-[(2-Chloroethyl)-2,4-dinitro-6-[[[2-(phosphonooxy)ethyl]amino]-  
carbonyl]anilino]ethyl methanesulfonate;

2-[(2-[Bis(2-bromopropyl)amino]-3,5-dinitrobenzoyl]amino)ethyl dihydrogen

phosphate;

2-[(2-Bromoethyl)-2,4-dinitro-6-[[[2-(phosphonooxy)ethyl]amino]-  
carbonyl]anilino]ethyl methanesulfonate;

2-[[2-[Bis(2-iodoethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl dihydrogen

phosphate;

2-[(2-Iodoethyl)-2,4-dinitro-6-([2-(phosphonooxy)ethyl]amino)carbonyl]-  
anilino]ethyl methanesulfonate;

2-[(2-Chloroethyl)-2,4-dinitro-3-[[[3-(phosphonooxy)propyl]amino]-  
carbonyl]anilino]ethyl methanesulfonate;

3-[(3-[Bis(2-bromoethyl)amino]-2,6-dinitrobenzoyl]amino)propyl dihydrogen  
phosphate;

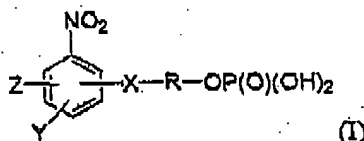
2-[(2-Bromoethyl)-2,4-dinitro-3-[[[2-(phosphonooxy)ethyl]amino]-  
carbonyl]anilino]ethyl methanesulfonate;

2-[(2-Bromoethyl)-2,4-dinitro-3-[[[3-(phosphonooxy)propyl]amino]-  
carbonyl]anilino]ethyl methanesulfonate; and

2-[(2-Iodoethyl)-2,4-dinitro-3-[[[3-(phosphonooxy)propyl]amino]-  
carbonyl]anilino]ethyl methanesulfonate.

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4 (currently amended). A method of preparing a phosphate represented by the general formula (I);



wherein:

X represents at any available ring position —CONH—, —SO<sub>2</sub>NH—, —O—, —CH<sub>2</sub>—, —NHCO— or —NHSO<sub>2</sub>—;

R represents a lower C<sub>1-6</sub> alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom;

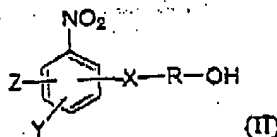
Y represents at any available ring position —N-aziridinyl or —N(CH<sub>2</sub>CH<sub>2</sub>W)<sub>2</sub>, where each W is independently selected from halogen or —OSO<sub>2</sub>Me;

Z represents at any available ring position —NO<sub>2</sub>—, —halogen—, —CN—, —CF<sub>3</sub> or —SO<sub>2</sub>Me;

and pharmaceutically acceptable salts and derivatives thereof;

the method ~~including~~ comprising the step of

(i) phosphorylating a compound of formula (II)



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wherein:

X represents at any available ring position  $-\text{CONH}-$ ,  $-\text{SO}_2\text{NH}-$ ,  $-\text{O}-$ ,  $-\text{CH}_2-$ ,  $-\text{NHCO}-$  or  $-\text{NHSO}_2-$ ;

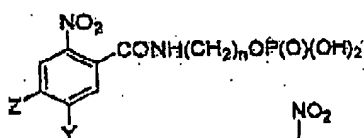
Y represents at any available ring position  $-\text{N-aziridinyl}$ ,  $-\text{N}(\text{CH}_2\text{CH}_2\text{W})_2$ , or  $-\text{N}(\text{CH}_2\text{CH MeW})_2$  where each W is independently selected from halogen or  $-\text{OSO}_2\text{Me}$ ;

Z represents at any available ring position  $-\text{NO}_2$ ,  $-\text{halogen}$ ,  $-\text{CN}$ ,  $-\text{CF}_3$  or  $-\text{SO}_2\text{Me}$ ; and

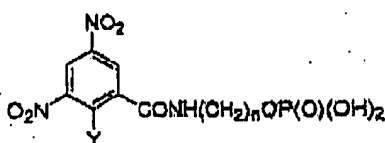
R represents a lower  $\text{C}_{1-6}$  alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom.

5 (currently amended). A method of preparing a compound of formulae (la),

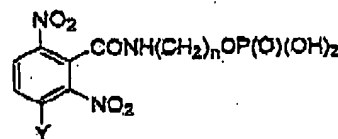
(lb) or (lc)



(la)

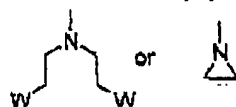


(lb)



(lc)

wherein Y may represent

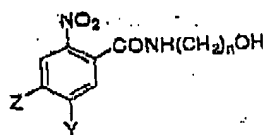


and wherein

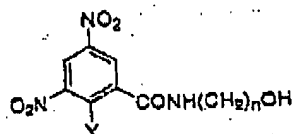
n represents 1 to 6

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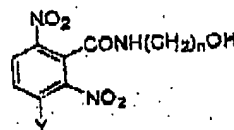
Z represents  $-\text{NO}_2$ ,  $-\text{halogen}$ ,  $-\text{CN}$ ,  $-\text{CF}_3$  or  $-\text{SO}_2\text{Me}$ ; and  
 where each W is independently selected from halogen or  $-\text{OSO}_2\text{Me}$   
 and pharmaceutically acceptable salts and derivatives thereof  
 the method including comprising the step of  
 phosphorylating a compound represented by formulae (IIa), (IIb) or (IIc)



(IIa)

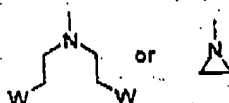


(IIb)



(IIc)

wherein Y represents



and wherein

n represents 1 to 6

Z represents  $-\text{NO}_2$ ,  $-\text{halogen}$ ,  $-\text{CN}$ ,  $-\text{CF}_3$  or  $-\text{SO}_2\text{Me}$ ; and

where each W is independently selected from halogen or  $-\text{OSO}_2\text{Me}$   
 and pharmaceutically acceptable salts and derivatives.

6-7 (canceled).

8 (currently amended). A method of anticancer treatment including  
comprising the step of administering an amount of a compound of Formula (I) as

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defined above in claim 1 to a subject.

9 (currently amended). A method of killing hypoxic cells in a tumour ~~including~~ comprising the step of administering an amount of a compound of Formula (I) as defined above in claim 1 to a subject with the tumour.

10 (previously presented). The method as claimed in claim 8 including the further step of applying irradiation or one or more chemotherapeutic agents to the subject.

11 (previously presented). The method as claimed in claim 8 wherein the subject is a human.

12 (previously presented). The method as claimed in claim 8 wherein the amount administered is between about 20% to 100% of the maximum tolerated dose of the subject.

13 (currently amended). A method of cell ablation utilising at least one nitroreductase enzyme ~~including~~ comprising the step of using a compound of Formula (I) as defined above in claim 1 in an effective amount to ablate cells which express at least one nitroreductase enzyme.

14 (currently amended). A method of cell ablation utilising at least one nitroreductase enzyme ~~including~~ comprising the step of administering a compound of

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Formula (I) as defined above in claim 1 in an effective amount to a subject to ablate cells which express at least one nitroreductase enzyme.

15 (original). The method as claimed in claim 14 wherein the at least one nitroreductase enzyme is encoded for by the *nfsB* gene of either *E. coli* or by orthologous genes in *Clostridia* species.

16 (previously presented). The method as claimed in claim 14 wherein the cells that express the at least one nitroreductase enzyme are tumour cells in tissue in the subject.

17 (previously presented). The method as claimed in claim 14 wherein the cell ablation is achieved through GDEPT (gene-directed enzyme-prodrug therapy).

18 (previously presented). The method as claimed in claim 14 wherein the cell ablation is achieved through ADEPT (antibody-directed enzyme-prodrug therapy).

19 (previously presented). The method as claimed in claim 14 wherein the cells are mammalian.

20 (previously presented). The method as claimed in claim 14 wherein the amount administered is between about 20% to 100% of the maximum tolerated dose of the subject.



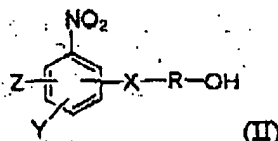
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21 (previously presented). The method as claimed in claim 14 including the further step of applying irradiation or one or more chemotherapeutic agents to the subject.

22 (currently amended). A pharmaceutical composition ~~including~~ comprising a therapeutically effective amount of a compound of Formula (I) as defined in claim 1 and a pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser.

23-26 (canceled).

27 (withdrawn). An alcohol compound of Formula (II)



wherein:

X represents at any available ring position -CONH-, -SO<sub>2</sub>NH-, -O-, -CH<sub>2</sub>-, -NHCO- or -NHSO<sub>2</sub>-;

Y represents at any available ring position -N-aziridinyl, -N(CH<sub>2</sub>CH<sub>2</sub>W)<sub>2</sub>, or -N(CH<sub>2</sub>CH MeW)<sub>2</sub> where each W is independently selected from halogen or -OSO<sub>2</sub>Me;

Z represents at any available ring position -NO<sub>2</sub>, -halogen, -CN, -CF<sub>3</sub> or -SO<sub>2</sub>Me;

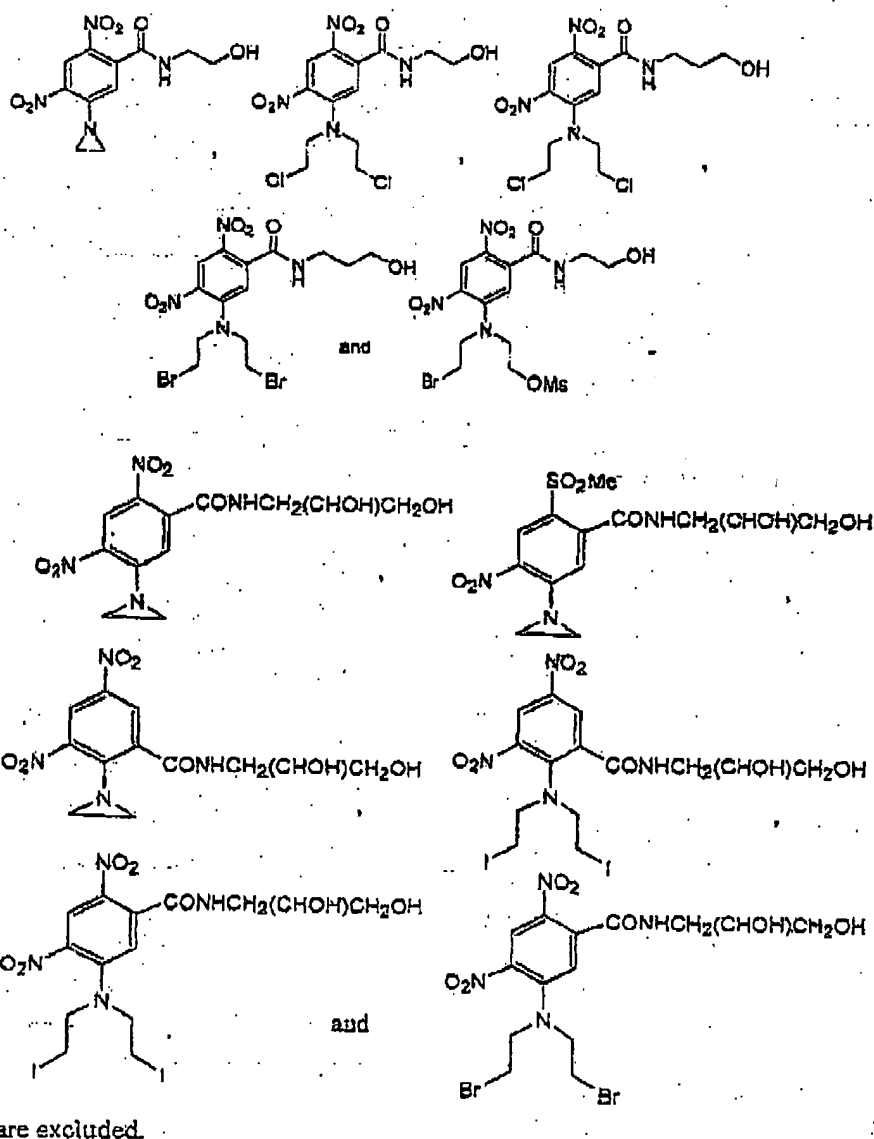
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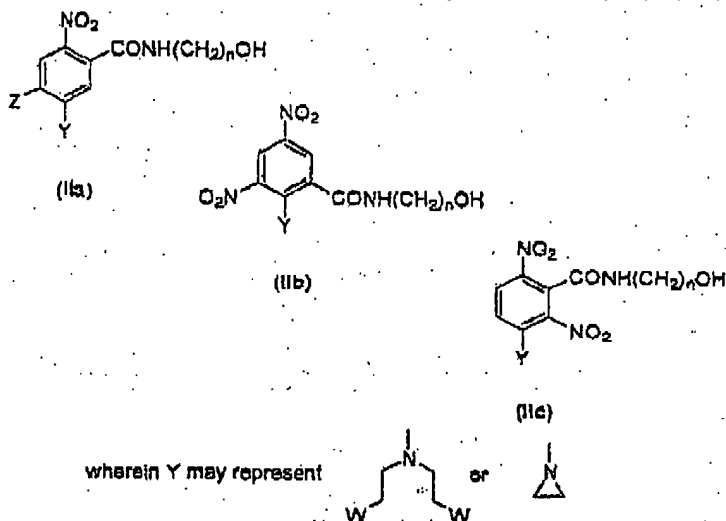
R represents a lower  $C_{1-6}$  alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and pharmaceutically acceptable salts and derivatives thereof; with the proviso that

when Z represents  $NO_2$  and Y represents  $N(CH_2CH_2C1)_2$ , X and R together cannot represent  $-CONHCH_2(CHOH)CH_2-$  and with the further proviso that the following compounds



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28 (withdrawn). The alcohol compound of Formula (II) as claimed in claim 27 selected from a compound represented by formulae (IIa), (IIb) or (IIc)



and wherein

n represents 1 to 6

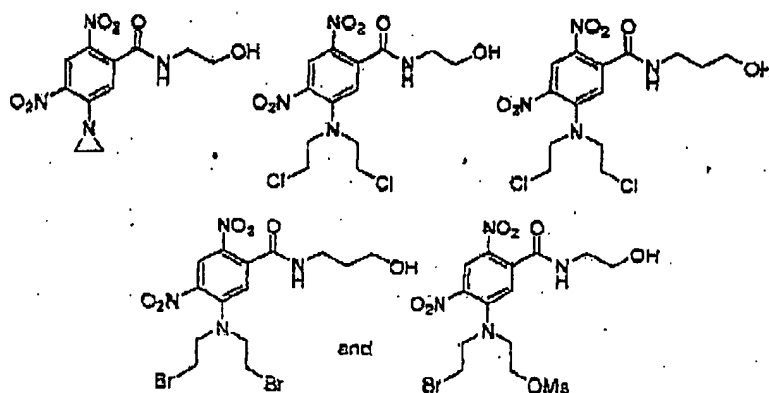
Z represents  $-\text{NO}_2$ ,  $-\text{halogen}$ ,  $-\text{CN}$ ,  $-\text{CF}_3$  or  $-\text{SO}_2\text{Me}$ ; and

where each W is independently selected from halogen or  $-\text{OSO}_2\text{Me}$  and

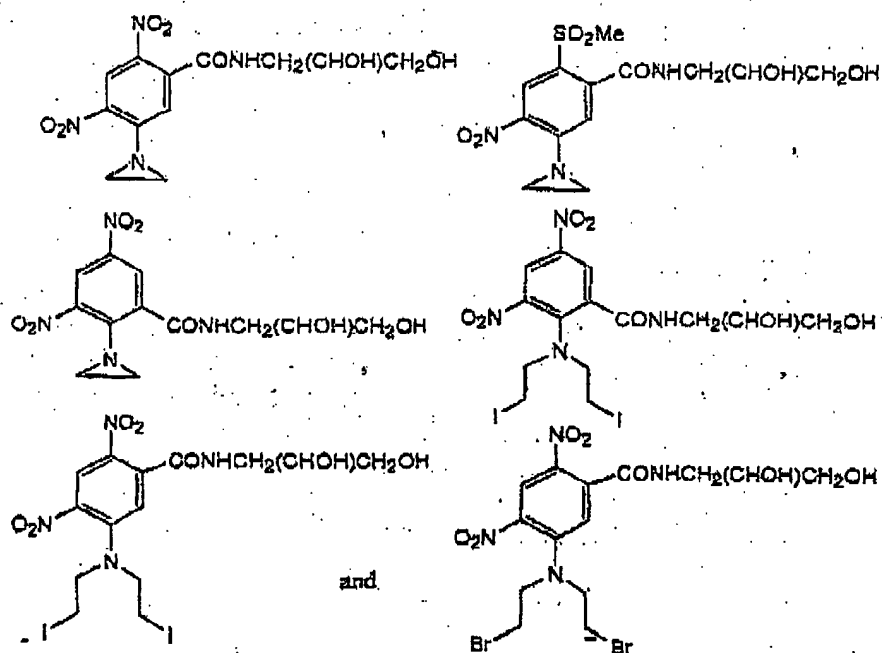
pharmaceutically acceptable salts and derivatives thereof with the proviso that

when Z represents  $\text{NO}_2$  and Y represents  $\text{N}(\text{CH}_2\text{CH}_2\text{Cl})_2$ , X and R together

cannot represent  $-\text{CONHCH}_2(\text{CHOH})\text{CH}_2-$  and with the further proviso that the following compounds



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are excluded.

29. (withdrawn). The alcohol compound of Formula (II) selected from a compound of Formula (IIb) or (IIc) as defined in claim 28.

30. (withdrawn). The alcohol compound of Formula (II) as defined in claim 28 selected from:

N-(2-Hydroxyethyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;

N-(4-Hydroxybutyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;

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N-(5-Hydroxypentyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;

N-(6-Hydroxyhexyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;

5-[Bis(2-bromoethyl)amino]-N-(2-hydroxyethyl)-4-(methylsulfonyl)-2-nitrobenzamide;

2-[(2-Bromoethyl)-5-[[[(3-hydroxypropyl)amino]carbonyl]-2,4-dinitroanilino]ethyl methanesulfonate;

5-[Bis(2-iodoethyl)amino]-N-(2-hydroxyethyl)-2,4-dinitrobenzamide;

2-[Bis(2-Chloroethyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide;

2-[Bis(2-bromoethyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide;

2-[Bis(2-chloroethyl)amino]-N-(3-hydroxypropyl)-3,5-dinitrobenzamide;

2-[Bis(2-bromoethyl)amino]-N-(3-hydroxypropyl)-3,5-dinitrobenzamide;

2-[Bis(2-chloroethyl)amino]-N-(4-hydroxybutyl)-3,5-dinitrobenzamide;

2-[Bis(2-bromoethyl)amino]-N-(4-hydroxybutyl)-3,5-dinitrobenzamide;

2-[Bis(2-chloroethyl)amino]-N-(5-hydroxypentyl)-3,5-dinitrobenzamide;

2-[Bis(2-bromoethyl)amino]-N-(5-hydroxypentyl)-3,5-dinitrobenzamide;

2-[Bis(2-chloroethyl)amino]-N-(6-hydroxyhexyl)-3,5-dinitrobenzamide;

2-[Bis(2-bromoethyl)amino]-N-(6-hydroxyhexyl)-3,5-dinitrobenzamide;

2-[Bis(2-bromopropyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide;

2-((2-Bromoethyl)-2-[[[(2-hydroxypropyl)amino]carbonyl]-4,6-dinitroanilino]ethyl methanesulfonate;

2-((2-Bromoethyl)-2-[[[(2-hydroxyethyl)amino]carbonyl]-4,6-dinitroanilino]ethyl methanesulfonate;

2-((2-Chloroethyl)-2-[[[(2-hydroxyethyl)amino]carbonyl]-4,6-dinitroanilino]ethyl

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methanesulfonate;

2-[Bis(2-iodoethyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide;

2-((2-iodoethyl)-2-(((2-hydroxyethyl)amino)carbonyl)-4,6-dinitroanilino)ethyl

methanesulfonate;

3-[Bis(2-bromoethyl)amino]-N-(2-hydroxyethyl)-2,6-dinitrobenzamide;

2-((2-bromoethyl)-3-(((2-hydroxyethyl)amino)carbonyl)-2,4-dinitroanilino)ethyl

methanesulfonate;

3-[Bis(2-bromoethyl)amino]-N-(3-hydroxypropyl)-2,6-dinitrobenzamide;

2-((2-bromoethyl)-3-(((3-hydroxypropyl)amino)carbonyl)-2,4-dinitroanilino)ethyl

methanesulfonate;

3-[Bis(2-bromoethyl)amino]-N-(4-hydroxybutyl)-2,6-dinitrobenzamide;

2-((2-bromoethyl)-3-(((4-hydroxybutyl)amino)carbonyl)-2,4-dinitroanilino)ethyl

methanesulfonate;

2-((2-chloroethyl)-3-(((3-hydroxypropyl)amino)carbonyl)-2,4-dinitroanilino)ethyl

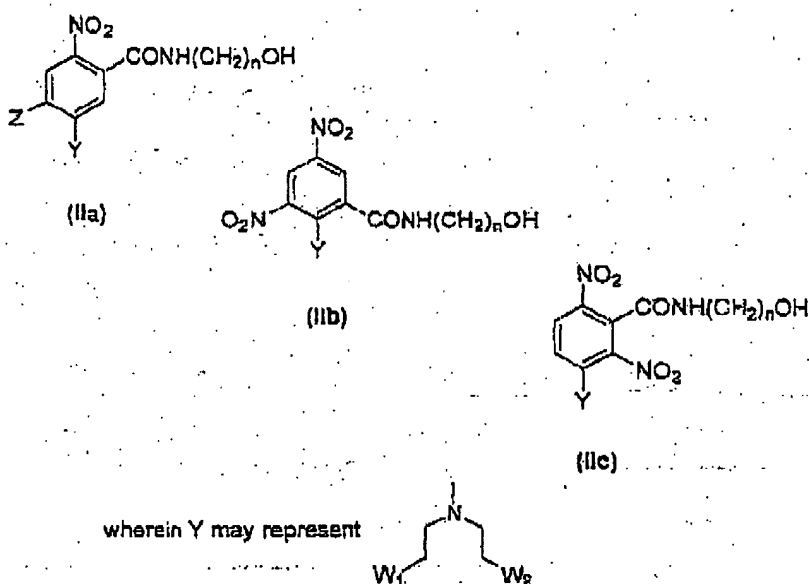
methanesulfonate; and

2-((2-iodoethyl)-3-(((3-hydroxypropyl)amino)carbonyl)-2,4-dinitroanilino)ethyl

methanesulfonate.

31 (withdrawn). A method of preparing a compound of formulae (IIa), (IIb) or (IIc)

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and wherein

$n$  represents 1 to 6

$\text{Z}$  represents  $-\text{NO}_2$ ,  $-\text{halogen}$ ,  $-\text{CN}$ ,  $-\text{CF}_3$  or  $-\text{SO}_2\text{Me}$ ; and

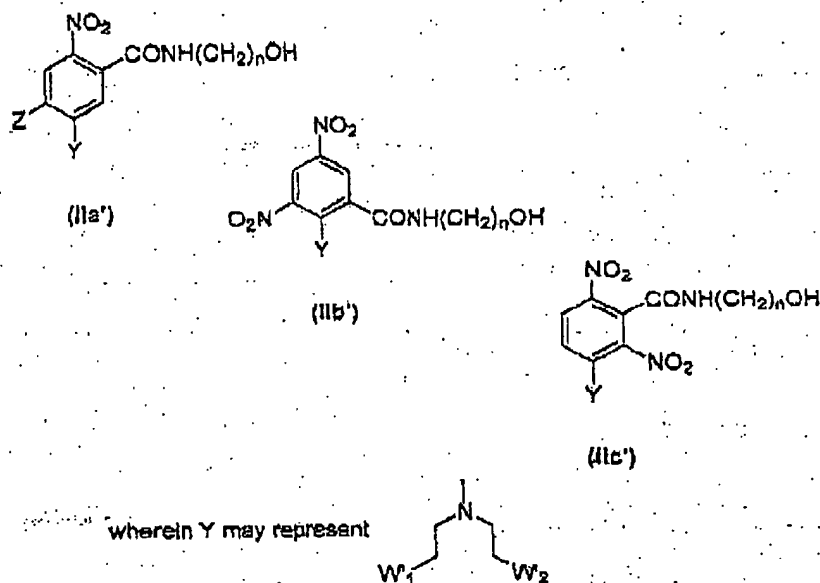
where  $\text{W}_1$  is halogen and  $\text{W}_2$  is  $-\text{OSO}_2\text{Me}$

and pharmaceutically acceptable salts and derivatives thereof;

the method including the step of

reacting a compound of formulae (IIa'), (IIb') or (IIc') optionally with heating

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wherein W'1 and W'2 are each halogen;

with an effective amount of silver methanesulfonate (AgOMs) in a solvent to give a compound of formulae (IIa), (IIb) or (IIc) defined above in this claim.

32 (withdrawn). The method as claimed in claim 31 wherein the solvent is selected from MeCN or other polar non-protic solvent.

33. (withdrawn). A compound of formula (IIa), (IIb) or (IIc) obtained by the method defined in claim 31.

34 (withdrawn). A method of anticancer treatment including the step of administering an amount of a compound of Formula (II) as defined in claim 27 to a



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subject.

35 (withdrawn). A method of killing hypoxic cells in a tumour including the step of administering an amount of a compound of Formula (II) as defined in claim 27 to a subject with the tumour,

36 (withdrawn). The method as claimed in claim 34 including the further step of applying irradiation or one or more chemotherapeutic agents to the subject.

37 (withdrawn). The method as claimed in claim 34 wherein the subject is a human.

38 (withdrawn). A method of cell ablation utilising at least one nitroreductase enzyme including the step of using a compound of Formula (II) as defined in claim 27 in an effective amount to ablate cells which express at least one nitroreductase enzyme.

39 (withdrawn). A method of cell ablation utilising at least one nitroreductase enzyme including the step of administering a compound of Formula (II) as defined in claim 27 in an effective amount to a subject to ablate cells which express at least one nitroreductase enzyme.

40 (withdrawn). The method as claimed in claim 39 wherein the at least one nitroreductase enzyme is encoded for by the *nfsB* gene of either *E. coli* or by

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orthologous genes in *Clostridia* species.

41 (withdrawn). The method as claimed in claim 39 wherein the cells that express the at least one nitroreductase enzyme are tumour cells in tissue in the subject.

42 (withdrawn). The method as claimed in claim 39 wherein the cell ablation is achieved through GDEPT (gene-directed enzyme-prodrug therapy).

43 (withdrawn). The method as claimed in claim 39 wherein the cell ablation is achieved through ADEPT (antibody-directed enzyme-prodrug therapy).

44 (withdrawn). The method as claimed in claim 39 wherein the cells are mammalian.

45 (withdrawn). The method as claimed in claim 39 including the thither step of applying irradiation or one or more chemotherapeutic agents to the subject.

46 (withdrawn). A pharmaceutical composition including a therapeutically effective amount of a compound of Formula (II) as claimed in claim 27 and a pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser.

47 (withdrawn). The use in the manufacture of a medicament of an effective amount of a compound of Formula (II) as claimed in claim 27 as an anticancer agent in

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a subject.

48 (withdrawn). The use as claimed in claim 47 wherein the medicament is further adapted for use in cell ablation in conjunction with at least one nitroreductase enzyme including GDEPT (gene-directed enzyme-prodrug therapy) or ADEPT (antibody-directed enzyme therapy).

49 (withdrawn). The use as claimed in claim 48 wherein the at least one nitroreductase enzyme is encoded for by the *nfsB* gene of either *E. coli* or by orthologous genes in *Clostridia* species.

50 (withdrawn). The use as claimed in claim 47 wherein the medicament is adapted for a mammalian subject.

51 (previously presented). A compound selected from:

2-[(2-Bromoethyl)-2,4-dinitro-6-[[[2-(phosphonooxy)ethyl]amino]-carbonyl]anilino]ethyl methanesulfonate;

2-[(2-Bromoethyl)-2,4-dinitro-3-[[[2-(phosphonooxy)ethyl]amino]-carbonyl]anilino]ethyl methanesulfonate;

2-[(2-Bromoethyl)-2,4-dinitro-3-[[[3-(phosphonooxy)propyl]amino]-carbonyl]anilino]ethyl methanesulfonate; and

2-[(2-Iodoethyl)-2,4-dinitro-3-[[[3-(phosphonooxy)propyl]amino]-carbonyl]anilino]ethyl methanesulfonate.

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52 (previously presented). The compound

2-[(2-Bromoethyl)-2,4-dinitro-6-[[[2-(phosphonooxy)ethyl]amino]-  
carbonyl]anilino]ethyl methanesulfonate.

53 (currently amended). A pharmaceutical composition ~~including~~ comprising a  
therapeutically effective amount of a compound as claimed in claim 51 or claim 52 and  
a pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser.

54-64 (cancelled).

65 (new). A method of treating cancer comprising the step of administering an  
effective amount of the compound of claim 52 to a subject.

66 (new). A method of killing hypoxic cells in a tumor comprising the step of  
administering an effective amount of a compound of claim 52 to a subject with a tumor.